Polyamines as Endogenous Modulators of the *N*-Methyl-D-Aspartate Receptor

EDYTHE D. LONDON[a–d] AND ALEXEY MUKHIN[a,e]

[a]*Neuroimaging and Drug Action Section*
Intramural Research Program
National Institute on Drug Abuse
National Institutes of Health
Baltimore, Maryland 21224

[b]*Department of Radiology*
Johns Hopkins Medical Institutions
Baltimore, Maryland 21205

[c]*Department of Pharmacology and Experimental Therapeutics*
School of Medicine
University of Maryland
Baltimore, Maryland 21201

Within the context of receptor interactions, ligand-gated ion channels are of particular interest because their activity is governed by signals from a variety of modulatory sites, each of which contributes to coordinated function. In this regard, the *N*-methyl-D-aspartate (NMDA) receptor is a multisubunit structure that bears binding sites for agonists and antagonists.[1] Both glutamate and glycine are required to effect receptor function, which is gating of a cationic channel that can be blocked by noncompetitive antagonists, such as phencyclidine, 1-[1-(2-thienyl)cyclohexyl]piperidine (TCP), and dizocilpine. In fact, function of the channel has been evaluated by assays of the binding of radiolabeled noncompetitive antagonists. Some of these studies revealed cooperative interactions between glutamate, glycine, and spermidine, suggesting that these modulators act at distinct, but allosterically coupled sites on the receptor.[2]

Polyamines are ubiquitous molecules that have been implicated in a variety of cellular functions, including growth and development, biosynthesis of nucleic acids and proteins, and regulation of mitochondrial Ca^{2+}.[3] They are formed via a pathway that involves the decarboxylation of ornithine to form putrescine. Subsequent steps involve biosynthesis of the charged triamine and tetramine, spermidine and spermine, respectively. Positive charges on polyamine molecules presumably could facilitate their interactions with various important biologically important sites, such as negatively charged moieties of nucleic acids and phospholipids of membranes.

Evidence for a neuromodulatory role of polyamines derives from a variety of sources.[4] Polyamines are present in brain at high concentrations (about 400 μM);[3,5,6] they influence behavioral indices of central nervous system excitability[7] and they inhibit the synaptosomal uptake of choline and dopamine.[8] Furthermore, spermidine is transported axonally in sciatic nerves,[9] and studies of cortical slices have revealed a high-affinity uptake system and Ca^{2+}-stimulated release of polyamines.[10] Release of

[d]Address correspondence to Edythe D. London, Ph.D., Chief, Neuroimaging and Drug Action Section, NIDA Division of Intramural Research, P.O. Box 5180, Baltimore, MD 21224.
[e]Present address: Departments of Neurology and Pharmacology, Georgetown University, Washington, DC 20007.

polyamines from synaptosomes by a high concentration of KCl also has been observed.[11]

Specific interactions of polyamines with the NMDA receptor have been observed both biochemically and electrophysiologically. Evidence that polyamines facilitate NMDA receptor activation is evidenced by observations that they enhance binding of [^{3}H]dizocilpine and [^{3}H]TCP to sites within the NMDA receptor channel.[12–16] Supporting data, obtained in physiological recordings from *Xenopus* oocytes, expressing NMDA receptor, have revealed that spermine potentiates responses to NMDA.[5,17,18]

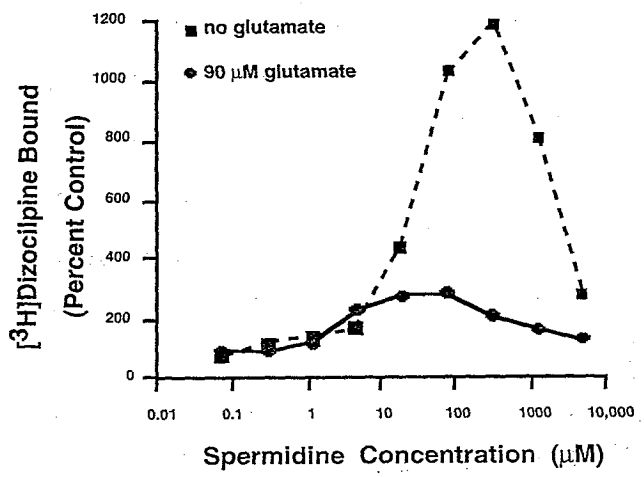

FIGURE 1. Enhancement of [^{3}H]dizocilpine binding by spermidine. Well-washed membranes (7 washes, 15 μg protein) obtained from rat forebrain (frontal cortex + hippocampus) were incubated with 2 nM [^{3}H]dizocilpine in 0.3 mL 10 mM HEPES · KOH buffer, pH 7.4, containing 30 μM glycine at 20 °C. Incubations were run for 6 h in the absence or presence of 30 μM added L-glutamate. Binding was terminated by filtration through GF/B filters presoaked in 0.5% polyethyleneimine. Nonspecific binding was determined in the presence of 1 μM cold, unlabeled dizocilpine. Each point represents the average specific binding, expressed as percent control, of triplicate determinations from one experiment. Control specific binding of [^{3}H]dizocilpine was 0.13 ± 0.01 pmol/mg protein and 0.83 ± 0.05 pmol/mg protein, the absence or presence of 90 μM added L-glutamate, respectively (mean ± SEM for triplicates). Similar results were obtained in two additional experiments.

The mechanisms by which polyamines apparently facilitate opening of the NMDA receptor channel have been investigated by assessing their effects on the binding of [^{3}H]dizocilpine under various conditions.[19] In most of these studies, spermine and spermidine stimulate [^{3}H]dizocilpine binding.[12,21–23] Without added glutamate, the stimulation is substantial (about 12-fold in our studies), at the maximally effective concentration of spermidine (about 500 μM) (FIG. 1). This effect could at least in part be accounted for by an enhancement in the affinity of the NMDA receptor for agonist. Nonetheless, spermidine also produces stimulation in the presence of a saturating concentration of glutamate[24] (FIG. 1), although the

stimulation is much smaller in magnitude. This stimulation could not reflect an increased affinity for the agonist, and suggests that spermidine exerts a positive action on the response to agonist binding, facilitating agonist-induced channel opening.

Although the putative mechanism by which polyamines may alter the functional response to agonist binding, as evidenced by increased binding of noncompetitive agonists, is not clarified, direct evidence is available regarding the effects of polyamines on NMDA recognition sites. In this regard, polyamines enhance the binding of [^{3}H](+)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid ([^{3}H]CPP), a competitive antagonist of NMDA or glutamate at these sites.[13] A similar action has been observed on the binding of [^{3}H]CGP 39653, another competitive antagonist that is highly selective for NMDA recognition sites[25] (see FIG. 2).

The ability to stimulate [^{3}H]CGP 39653 binding appears to be a property common to all polyamines, as it is observed when incubations are performed with spermine and putrescine as well as with spermidine (FIG. 2). The rank order of potencies of the amines is inversely related to the number of amino groups in the molecule, indicating that the number of positive charges determines potency.

The view that polyamines enhance binding to NMDA recognition sites by an enhancement of affinity for the radioligands is supported by a recent report in which spermine reduced the K_d for [^{3}H]CGP 39653 binding from about 15 nM to 3 nM.[25] However, our studies of [^{3}H]CGP 39653 binding to well-washed membranes of rat forebrain indicated behavior consistent with a two-site model (FIG. 3), and with the existence of two states of NMDA recognition sites, exhibiting K_d values of approximately 6 nM and 200 nM for high- and lower-affinity sites, respectively. Furthermore, as the high-affinity sites represented a small fraction (about 25%) of the total density

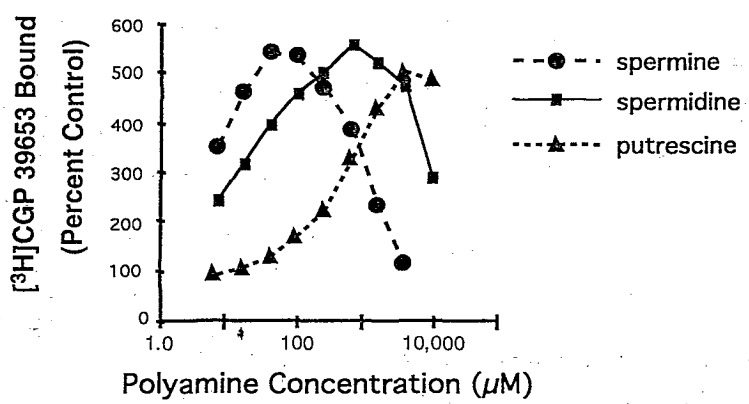

FIGURE 2. Stimulation of [^{3}H]CGP 39653 binding by polyamines. Well-washed membranes of rat forebrain (24 μg protein) were incubated with 3.2 nM [^{3}H]CGP 39653 in 0.3 mL of 10 mM HEPES · KOH buffer, pH 8.0, containing various concentrations of polyamines. Incubations were run for 1 h at 4 °C. Binding was terminated by filtration through GF/B filters presoaked in 0.5 M Tris HCl, pH 8.0, containing 1 M KCl and 0.1 mM glutamate. Nonspecific binding was determined in the presence of 100 μM NMDA. Each point represents the average specific binding, expressed as percent control, of triplicate determinations from one experiment. Specific binding of [^{3}H]CGP 39653 in the absence of added polyamines (control) was 0.51 ± 0.03 pmol/mg protein (mean ± SEM for triplicates). Similar results were obtained in two to four additional experiments.

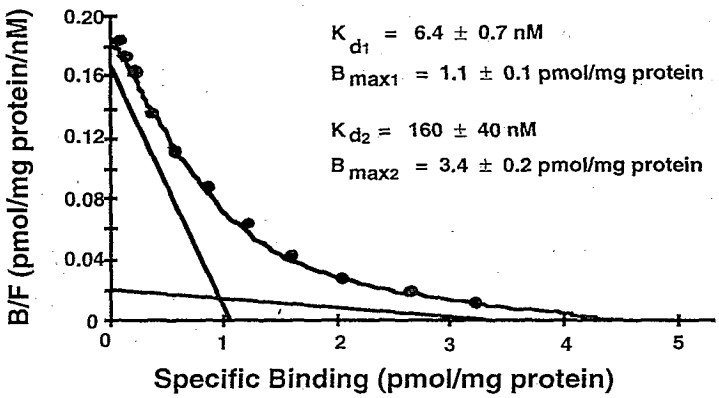

FIGURE 3. Scatchard analysis of [^{3}H]CGP 39653 binding to well-washed membranes from rat forebrain. Membranes (18 μg protein) were incubated with each of 11 concentrations of [^{3}H]CGP 39653 (0.3–300 nM) in 0.3 mL 10 mM HEPES · KOH buffer, pH 8.0, at 4 °C for 1 h. Nonspecific binding was determined in the presence of 500 μM NMDA. Binding was terminated by filtration through GF/B filters presoaked in 0.5 M Tris HCl pH 8.0, containing 1 M KCl and 0.1 mM glutamate. Each point represents the mean of 16 replicates (quadruplicate determinations from four separate assays performed on the same tissue preparation). Values of K_d and B_{max} indicate the means and standard errors obtained from a single nonlinear, least-squares regression analysis of the data from this experiment. Similar results were obtained in eight additional assays.

of specific binding sites in rat forebrain, additional studies of polyamine interactions with NMDA recognition sites seemed warranted.

Saturation studies of [^{3}H]CGP 39653 binding, therefore, were performed in the absence of added polyamines, and were compared to data obtained when 500 μM spermidine was added to the incubations (FIG. 4). The concentration of spermidine was selected on the basis of its ability to induce an approximately fivefold increase in the binding of [^{3}H]CGP 39653 (FIG. 2). When spermidine was added, Scatchard analysis of the resultant data did not support a two-site model, because only one population of sites was apparent (FIG. 4B). The affinity of the sites corresponded closely to that of the high-affinity portion of binding obtained in the absence of added spermidine (FIGS. 3 and 4A). Furthermore, in the presence of spermidine, the density of sites (B_{max}) was nearly equal to the sum of the densities of high- and low-affinity sites assayed in the control condition, and close to the density of sites assayed with [^{3}H]dizocilpine (about 5 pmol/mg protein). Therefore, it appeared that the major reason for the spermidine-induced enhancement of [^{3}H]CGP 39653 binding was not to increase the affinity of the sites, but rather to convert the low-affinity sites to a high-affinity conformation. Nonetheless, the present data suggest that spermidine may also produce a slight enhancement in affinity of the high-affinity sites.

Competition assays revealed that the sites converted by spermidine from a low- to a high-affinity conformation had a pharmacological profile consistent with that of NMDA recognition sites, and similar or identical to that of high-affinity sites labeled in the absence of added spermidine. Under both conditions, ligands for NMDA recognition sites (NMDA, glutamate, CPP, and (±)2-amino-5-phosphonopentanoic

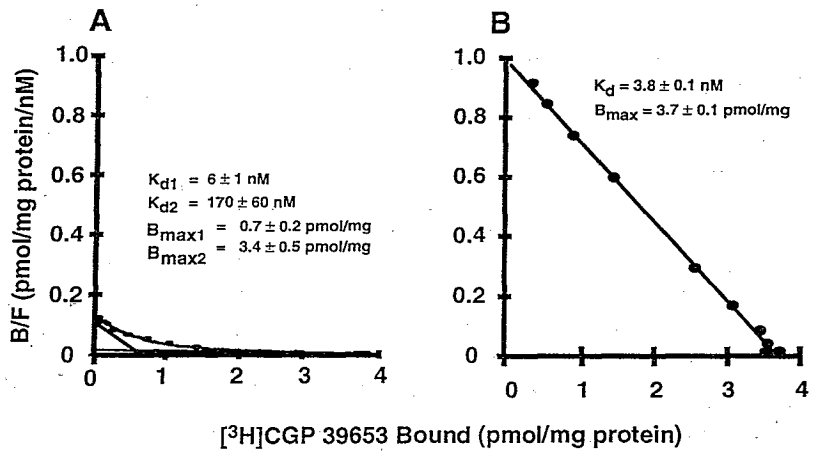

FIGURE 4. Effect of spermidine on parameters of [^{3}H]CGP 39653 binding in well-washed membranes of rat forebrain (see FIG. 3 legend for assay procedure). Results are shown of an experiment performed in quadruplicate using the same membrane preparation in the absence (**A**) and presence (**B**) of 500 μM spermidine. Values of K_d and B_{max} are the means ± SEM for individual Scatchard analyses. Similar results were obtained in three additional experiments.

acid) had much higher potencies than ligands for non-NMDA glutamate binding sites (quisqualic acid, kainic acid, cystine, *trans*-1-amino-cyclopentyl-1,3-decarboxylate); and the potencies of the inhibitors in the absence of spermidine were highly correlated with corresponding potencies when spermidine was added.

The results of these studies are consistent with the view that polyamines modulate activity of the NMDA receptor by at least two mechanisms. One of these, inferred from the stimulation of [^{3}H]dizocilpine binding in the presence of saturating concentrations of glutamate, is independent of an action on affinity of NMDA recognition sites. Another mechanism of polyamine-induced activation of the NMDA receptor, as evidenced by an increase in the binding of noncompetitive antagonists to the channel, appears to involve an enhancement in the sensitivity of NMDA recognition sites. Although previous studies have indicated that this action reflects an increase in affinity of the sites, the present report demonstrates that a major aspect of the spermidine-induced increases in binding to these sites is through a conversion of NMDA recognition sites from a low-affinity conformation to one of high affinity. This action of polyamines is a function of the number of amine groups on the molecule and is shared by a variety of organic and inorganic cations.[26] Therefore, relatively nonspecific stimulatory effects of cations on the NMDA receptor could be relevant to receptor function *in vivo,* and this has implications for the interpretation of *in vitro* assays of NMDA receptors.

REFERENCES

1. NAKANISHI, S. 1992. Molecular diversity of glutamate receptors and implications for brain function. Science **258:** 597–603.
2. RANSOM, R. W. & N. L. STEC. 1988. Cooperative modulation of [^{3}H]MK-801 binding to the *N*-methyl-D-aspartate receptor-ion channel complex by L-glutamate, glycine, and polyamines. J. Neurochem. **51:** 830–836.

3. TABOR, C. W. & H. TABOR. 1984. Polyamines. Annu. Rev. Biochem. **53:** 749–790.
4. SHAW, G. G. 1979. The polyamines in the central nervous system. Biochem. Pharmacol. **28:** 1–6.
5. SEILER, N. & F. N. BOLKENIUS. 1985. Polyamine reutilization and turnover in brain. Neurochem. Res. **10:** 529–544.
6. SLOTKIN, T. A. & J. BARTOLOME. 1986. Role of ornithine decarboxylase and the polyamines in nervous system development: A review. Brain Res. Bull. **17:** 307–320.
7. ANDERSON, D. J., J. CROSSLAND & G. G. SHAW. 1975. The actions of sperimidine and spermine on the central nervous system. Neuropharmacology **14:** 571–577.
8. LAW, C.-L., P. C. L. WONG & W.-F. FONG. 1984. Effects of polyamines on the uptake of neurotransmitters by rat brain synaptosomes. J. Neurochem. **42:** 870–872.
9. LINDQUIST, T. D., J. A. STURMAN, R. M. GOULD & N. A. INGOGLIA. 1985. Axonal transport of polyamines in intact and regenerating axons of the rat sciatic nerve. J. Neurochem. **44:** 1913–1919.
10. HARMAN, R. J. & G. G. SHAW. 1981. High-affinity uptake of spermine by slices of rat cerebral cortex. J. Neurochem. **36:** 1609–1615.
11. GILAD, G. M. & V. H. GILAD. 1991. Polyamine uptake, binding and release in rat brain. Eur. J. Pharmacol. **193:** 41–46.
12. WILLIAMS, K., C. ROMANO & P. B. MOLINOFF. 1989. Effects of polyamines on the binding of [^{3}H]MK-801 to the *N*-methyl-D-aspartate receptor: Pharmacological evidence for the existence of a polyamine recognition site. Mol. Pharmacol. **36:** 575–581.
13. CARTER, C. J., K. G. LLOYD, B. ZIVKOVIC & B. SCATTON. 1990. Ifenprodil and SL 82.0715 as cerebral antiischemic agents. III. Evidence for antagonistic effects at the polyamine modulatory site within the *N*-methyl-D-aspartate receptor complex. J. Pharmacol. Exp. Ther. **253:** 475–482.
14. ANIS, N., S. SHERBY, R. GOODNOW, JR. *et al.* 1990. Structure-activity relationships of philanthotoxin analogs and polyamines on *N*-methyl-D-aspartate and nicotinic acetylcholine receptors. J. Pharmacol. Exp. Ther. **254:** 764–773.
15. STEELE, J. E., D. M. BOWEN, P. T. FRANCIS, A. R. GREEN & A. J. CROSS. 1990. Spermidine enhancement of [^{3}H]MK-801 binding to the NMDA receptor complex in human cortical membranes. Eur. J. Pharmacol. **189:** 195–200.
16. ROBINSON, T. N., C. ROBERTSON, A. J. CROSS & A. R. GREEN. 1990. Modulation of [^{3}H]-dizocilpine ([^{3}H]-MK-801) binding to rat cortical *N*-methyl-D-aspartate receptors by polyamines. Mol. Neuropharmacol. **1:** 31–35.
17. BRACKLEY, P. R., J. R. GOODNOW, K. NAKANISHI, H. L. SUDAN & P. N. R. USHERWOOD. 1990. Spermine and philanthotoxin potentiate excitatory amino acid responses of *Xenopus* oocytes injected with rat and chick brain RNA. Neurosci. Lett. **114:** 51–56.
18. MCGURK, J. F., M. V. L. BENNETT & R. S. ZUKIN. 1990. Polyamines potentiate responses of *N*-methyl-D-aspartate receptors expressed in *Xenopus* oocytes. Proc. Natl. Acad. Sci. USA **87:** 9971–9974.
19. WILLIAMS, K., C. ROMANO, M. A. DICHTER & P. B. MOLINOFF. 1991. Modulation of the NMDA receptor by polyamines. Life Sci. **48:** 469–498.
20. BAKKER, M. H. M., R. M. MCKERNAN, E. H. F. WONG & A. C. FOSTER. 1991. [^{3}H]MK-801 binding to *N*-methyl-D -aspartate receptors solubilized from rat brain: Effects of glycine site ligands, polyamines, ifenprodil, and desipramine. J. Neurochem. **57:** 39–45.
21. JAVITT, D. C., M. J. FRUSCIANTE & S. R. ZUKIN. 1994. Activation-related and activation-independent effects of polyamines on phencyclidine receptor binding within the *N*-methyl-D-aspartate receptor complex. J. Pharmacol. Exp. Ther. **270:** 604–613.
22. SUBRAMANIAM, S. & P. MCGONIGLE. 1991. Quantitative autoradiographic characterization of the binding of (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclo-hepten-5,10-imine ([^{3}H]MK-801) in rat brain: Regional effects of polyamines. J. Pharmacol. Exp. Ther. **256:** 811–819.
23. ROMANO, C., K. WILLIAMS & P. B. MOLINOFF. 1991. Polyamines modulate the binding of [^{3}H]MK-801 to the solubilized *N*-methyl-D-aspartate receptor. J. Neurochem. **57:** 811–818.
24. ROMANO, C., K. WILLIAMS, S. DEPRIEST, *et al.* 1992. Effects of mono-, di-, and triamines on

the *N*-methyl-D-aspartate receptor complex: A model of the polyamine recognition site. Mol. Pharmacol. **41:** 785–792.

25. REYNOLDS, I. J. 1994. [^{3}H]CGP 39653 binding to the agonist site of the *N*-methyl-D-aspartate receptor is modulated by Mg^{2+} and polyamines independently of arcaine-sensitive polyamine site. J. Neurochem. **62:** 54–62.
26. MUKHIN, A., E. S. KOVALEVA & E. D. LONDON. 1994. Effects of mono- and divalent cations on binding properties of NMDA recognition sites. Submitted.